allogeneic cell therapies, cell banks are essential both during preclinical development and manufacturing clinical products. The objective of this study is to establish a cell bank system for preclinical research of a cartilage repair biologic. METHODS/STUDY POPULATION: To conduct large animal preclinical studies required by the FDA for load-bearing cartilage repair products, the FDA has described in its guidance documents for Investigational New Drug (IND) applications the use of an animal-based "analogous cellular product" (ACP). Here, costal chondrocytes from 6 minipigs (3 male, 3 female) were isolated and expanded to create master cell banks (MCBs) at passage 2. ACPs (i.e., tissue-engineered neocartilage) were generated from each MCB, and the critical quality attributes, properties such as morphology and mechanics, were assessed. By comparing functional properties of the ACPs to those of the native cartilage tissue they intend to replace, donors were selected for inclusion in working cell banks (WCBs) to support both academic research and ongoing IND-enabling research. RESULTS/ANTICIPATED RESULTS: All six donors produced tissue-engineered cartilage that passed morphologic release criteria (i.e., round, flat). However, ACPs showed distinct donor-based variability in terms of mechanical release criteria (e.g., tensile Young's modulus and compressive aggregate modulus). Based on release criteria data, two types of WCBs were created at passage 5: 1) a pooled donor WCB for academic studies that require multiple donors and 2) multiple single-donor WCBs for manufacturing ACPs tested in ongoing IND-enabling dose-response and pivotal long-term studies. It is estimated that between 160k-400k, ACP constructs can be produced from each of the six MCBs, analogous to clinical-scale manufacturing. DISCUSSION/SIGNIFICANCE OF IMPACT: Overall, this study illustrates a cell bank system that can be used to support IND-enabling research. This cell bank produced preclinical neocartilage analogous to the intended clinical product, and it is currently being used in dose-response and pivotal long-term large animal studies for temporomandibular joint disc repair.

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Elucidating the function of muscle-resident macrophages in cancer cachexia<t>

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OBJECTIVES/GOALS: Cancer cachexia is a wasting condition common in pancreatic cancer, marked by loss of muscle, fat, and bone and driven by inflammation. Immune signals contribute to this damage. This study examines how muscle-resident macrophages may regulate muscle loss in pancreatic cancer cachexia. METHODS/STUDY POPULATION: To identify resident macrophages in cachectic muscle, Lyve1Cre-Rosa26;TdTomato mice were orthotopically transplanted with KPCML1 pancreatic cancer cells. Resident macrophages were analyzed via flow cytometry and immunohistochemistry. For muscle-specific macrophage ablation, lyve1-DTR mice (C57BL/6 background) received tumor cell injections followed by intramuscular diphtheria toxin (DTA) in the TA muscle. Quantitative RT-PCR assessed gene expression from whole muscle lysates and flow-sorted TdTomato+ cells. RESULTS/ANTICIPATED RESULTS: We show that the population of muscle-resident macrophages increased as tumor burden increased. Further findings reveal that the ablation of the resident population of macrophages exacerbates muscle loss, suggesting that muscle-resident macrophages exude a protective phenotype on skeletal myofibers. This was confirmed with increased expression of atrophy biomarkers, MuRF1 and Atrogin-1, in muscles from DTA-injected mice. Additionally, preliminary data revealed an expansion of Toll-like receptor 7 (TLR7) expression in muscle-resident macrophages, suggesting that TLR7 signaling may contribute to their phenotypic regulation in cachexia. DISCUSSION/SIGNIFICANCE OF IMPACT: Results suggest muscle-resident macrophages protect against cachexia-induced atrophy via anti-inflammatory activity. Future work will define their signals and targets. Given TLR7 upregulation, we will test if TLR7 signaling regulates their phenotype and contributes to muscle atrophy.

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Using participant-driven recruitment strategies to enhance clinical trial enrollment

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OBJECTIVES/GOALS: Clinical trial recruitment needs innovative strategies to increase efficiency. Participant-driven recruitment (PDR) strategies are an advancement of snowball sampling that have been tested in observational studies (e.g., as respondent-driven sampling) but are infrequently used in clinical trials. METHODS/STUDY POPULATION: In preparation for testing participant-driven recruitment as a clinical trial innovation, we conducted interviews with experts (principal investigators, project managers, and methodologists (e.g., biostatisticians)) involved in ongoing trials. The expert's trials included behavioral, screening, and pharmaceutical therapies. We described the PDR concept to our experts and asked what sorts of trials might be amenable to the approach. We asked experts to discuss when in the course of trial workflow would be ideal for engaging current participants to identify and refer others who might be interested and eligible; and what methods concerns might result from using PDR. RESULTS/ANTICIPATED RESULTS: Experts (n = 10) noted studying disease prevention or screening, or focused on a common condition (e.g., diabetes, hypertension) was most amenable to PDR, whereas rare disease trials would be challenging. Opinions differed regarding when in the enrollment process might be best to engage participants to refer others (time of consent versus after completion of active intervention). From a methods standpoint, common concerns were contamination (if socially connected individuals were randomized to different arms) and that participant-referred individuals would not be independent, thus requiring special statistical considerations. Most experts (8 of 10) expressed interest in learning best practices for PDR (e.g., incentivizing referrals, setting referral quotas and timelines) and implementing the process. DISCUSSION/

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